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Introduction

Thielavia basicola Zopf[2] is known to attack the roots of at least one hundred angiospermous plants (Johnson, 1916*a*). The root-rot of commercial tobacco (*Nicotiana tabacum*) caused by Thielavia is perhaps of greater economic importance than that on any other host plant. The fungus and the disease symptoms have been so well described by Gilbert (1909) that no further discussion of these points will be given here.

Since the first discovery of Thielavia on tobacco by Peglion (1897) a large amount of work has been done on this disease by various investigators. The general trend of their research has been toward practical control measures, although a considerable number of infection and environment experiments have been performed. Of the latter, the most critical investigation was that of Johnson and Hartman (1919). These workers, after a careful study of the effects, respectively, of amount of soil infestation, soil moisture, soil temperature, soil reaction, physical structure, and fertility, concluded that temperature is "the most important factor determining the effect of root-rot of tobacco," other conditions being equal. They found also that, disregarding other factors, the amount of damage is directly proportional to the amount of infestation in the soil. By using the Wisconsin soil temperature tanks (Jones, 1917) they determined that the temperature most favorable for the disease ranges from 17° to 23° C., and that the disease is reduced at temperatures from 26° upward, until at 32° C. practically no infection occurs even in the most heavily infested soils.

Previously, Gilbert (1909) had determined the optimum temperature for the development of the fungus in culture to be about 28° to 32° C., with 34° to 37° C. as the upper limit. The growth is relatively poor at 17° to 23° C. or lower. The optimum temperature for the growth of the host lies in approximately the same range as that of the fungus, although both host and parasite grow fairly well at 20° C. It would seem, therefore,

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

[2] A recent paper by Miss McCormick (1925) gives evidence that the conidial and ascigerous stages previously described together under this name may really be distinct forms. She retains the name *Thielavia basicola* Zopf for the perfect stage and applies the name *Thielaviopsis basicola* (Berk.) Ferraris to the conidial stage. Because of the general occurrence of the more inclusive term, *Thielavia basicola* Zopf, in the literature, this nomenclature will be adhered to in the present discussion.

that the reduction of the disease at 26° C. and above is probably due to host reaction alone, or at least primarily.

Johnson and Milton (1919) reported strains of White Burley tobacco resistant to root-rot. These strains stood up remarkably at temperatures at which no growth occurred in the common susceptible strains, both being grown in the same infested soil. Other strains, of varying resistance, have been discovered or developed in a number of localities (Johnson, 1916*b*).

Since little or no investigation on the histological aspect of tobacco root-rot had been reported in the literature up to 1925, the facts mentioned above seemed to present a unique opportunity for a study of the physical basis of disease resistance in this particular case. The problem under consideration may be stated as follows:

(1) To determine by histological methods whether or not the freedom from root-rot shown by susceptible tobacco at high temperatures is accompanied by any visible host reaction.

(2) To determine whether or not there is any correlation between host reaction and varietal resistance at lower temperatures, and if so whether such host reaction is of the same nature in this second type as in the first.

Methods

Inoculations

The original inoculum for this series of experiments was soil taken from an infested plot in the experimental fields of the Wisconsin Experiment Station. The infestation of the soil had been proved repeatedly by consistent infection of susceptible varieties of tobacco grown therein.

No attempt was made to secure pure-culture inoculum for these experiments. Isolations were made from lesions occurring on the roots of the susceptible Connecticut No. 38 tobacco which had been grown in the infested soil at 20° C. for four weeks. The fungus obtained from these isolations agreed very closely in measurements and general appearance with the *Thielavia basicola* described by Gilbert (1909). When a suspension of endoconidia from these isolations was poured upon sterile soil in which a healthy plant of susceptible White Burley tobacco was growing at 20° C., characteristic lesions bearing chlamydospores developed in nine days.

Seven varieties (or strains) of tobacco were used in these experiments. They were selected on the basis of their relative resistance to Thielavia as determined by Johnson's trials (1916*b*), which showed the White Burley and Maryland Broadleaf varieties to be very susceptible to Thielavia, while the Little Dutch variety was shown to be resistant. Unpublished data by the same investigator show that the Connecticut No. 38 strain and the Resistant White Burley strain really belong in a semi-resistant class, and that the Havana No. 142 strain falls approximately with Little Dutch in the resistant group. Xanthia, a strain of Turkish tobacco, was found to

be practically immune to Thielavia root-rot. Authentic seeds of all varieties used in these studies were secured from Johnson. The experimental results, therefore, are based on the use of a range of varieties (or strains) of tobacco from the most susceptible to the most resistant types known. These differences in terms of yield under field conditions often amount to a 1-to-100 ratio on infested soil. The seven strains are for convenience classified as follows:

Very susceptible: Maryland Broadleaf
Susceptible: White Burley
Resistant: Resistant White Burley; Connecticut No. 38
Very resistant: Little Dutch; Havana No. 142
Extremely resistant (practically immune): Xanthia

Plants of these strains were grown from seed in flats of steam-sterilized soil. At about the seven-leaf stage they were transplanted into the experimental culture cans, some containing infested soil, others with steam-sterilized soil. Two plants of each strain, one in infested soil and one in sterilized soil, in separate cans, were placed as follows: (1) in a tank held at 18°–20° C.; (2) in a greenhouse ranging from 22° to 25° C.; (3) in a tank held at 28°–30° C. In the first series Maryland Broadleaf, Susceptible Burley, Resistant Burley and Little Dutch were used. In the second series, Maryland Broadleaf and Little Dutch were replaced by Havana No. 142 and Xanthia. Connecticut No. 38 was grown separately, as a check, at 30° C. The air temperatures prevailing about the aërial parts of the plants did not vary more than 5° C. on either side of the soil temperatures.

All these experiments were run continuously at the same temperatures for six weeks. At the end of that time the plant heights were measured; the soil was washed from the roots with a fine spray of water; the condition of the root system as a whole was observed in each case; and, finally, portions of typical roots of various sizes from healthy plants and pieces with representative lesions from the infected plants were fixed and imbedded in paraffin.

Histological Technic

In the first series healthy and diseased root tissues were fixed in both Flemming's medium solution and in formalin-acetic-alcohol, Rawlins' formula (Rawlins and Johnson, 1925, p. 20). The formalin-acetic-alcohol was found to give satisfactory fixation of both host and fungus, and since it did not obscure the lesions by blackening and did not necessitate bleaching of the tissue subsequently, it was used alone for all fixations in the second series. All the fixations were imbedded in paraffin by a long schedule. Pieces of imbedded root which proved resistant to sectioning on the rotary microtome were trimmed in the paraffin block to expose the cut end of the root, and were soaked for from several days to several months in water to which a trace of formalin had been added to prevent the growth of

decay organisms.[3] The material in paraffin was sectioned 6–8 microns in thickness for the study of the fungus and of fungus invasion, and 10–15 microns for the histology of the host tissue. A modification of Flemming's triple stain was used on all sections after a number of stain combinations had been tried. This schedule stains lignified, suberized, cutinized and pectic walls red, fungus mycelium blue or orange, and cellulose walls and normal cytoplasm orange. Normal nuclear reticula take a deep blue stain, showing a gradual change to red in stages of disintegration.

Results

Gross Observations

The effect of fungal invasion on the growth of six strains of tobacco at various temperatures is shown in table 1. The height of the tobacco

Table 1. *Height in Inches of Six Varieties of Tobacco Grown for Six Weeks at Different Soil Temperatures in Infested and Uninfested Soil*

Host	Soil Temperatures					
	20° C.		25° C.		30° C.	
	Sterile	Infested	Sterile	Infested	Sterile	Infested
Series 1						
Maryland Broadleaf	6	2.0	17	3.0	—	—
Susceptible Burley	7	2.5	17	2.5	—	—
Resistant Burley	6	1.0	13	3.5	—	—
Little Dutch	14	8.0	25	11.0	—	—
Series 2						
Susceptible Burley	3.5	No growth	8	1.5	16	12
Resistant Burley	4.0	2	6	2.0	12	11
Havana No. 142	5.0	4	12	8.0	17	15
Xanthia	14.0	12	14	14.0	17	21

plant is a fairly accurate criterion of its resistance to Thielavia, allowing for natural varietal differences in modes of growth. Resistant Burley shows a tendency to produce a shorter, stockier growth than the other strains. Consequently its resistant qualities at the lower temperatures are not always evident from the plant heights.

Histological Observations

Anatomy of the Normal Tobacco Root (Pl. LXI, fig. 35)

The primary root structure of *Nicotiana tabacum*, as soon as any differentiation of tissues has taken place, consists of a uniseriate epidermis, a simple cortex, an endodermis with no thickenings in the tangential walls, a uniseriate pericycle, and several protoxylem groups alternating with an

[3] This softening process is the method suggested by Dr. Land of the University of Chicago and perfected by Dr. Kraus of the University of Wisconsin.

equal number of inconspicuous groups of protophloem cells. This root is often a millimeter or more in cross-sectional diameter, and may have a cortex several cells thick in main roots, that is, those originating at or near the crown of the plant. The fine branch roots, however, may be reduced to a minimum of two groups each of protoxylem and protophloem, a uniseriate pericycle, endodermis, cortex, and epidermal layer. In such roots the endodermis is by far the most conspicuous tissue by reason of its large cells. These branch roots are apparently rather ephemeral, being "pruned" off by subsequent growth of the main root. All the primary cells are nearly isodiametric in cross section, but are more or less elongated parallel to the axis of the root. The parenchymatous cells usually become stretched tangentially due to growth increases in the diameter of the root.

The epidermal cells develop hairs some distance back of the elongating region, and these structures may persist for some time. Before any secondary growth takes place, however, the hairs shrivel and the outer walls of the epidermal cells become thoroughly suberized and lignified. This layer may collapse and the next layer within become suberized and lignified, and the process may be repeated several times before secondary growth has proceeded far.

The cortex increases somewhat in size as the root matures, tangential divisions and enlargement of the cells apparently accommodating it for some time to the increasing diameter of the stele. The entire cortex disappears usually after a few weeks' growth of the root under normal conditions.

The endodermis is not conspicuous, at least in roots of average size, and can be recognized with certainty only by the presence of the narrow Casparian strips which stain deep red with safranin, and which appear as short, narrow rods in cross section, exactly adjacent on neighboring radial walls. The endodermis may accommodate itself to the growth of the stele for a short time, but it becomes almost unrecognizably collapsed before the cortex has entirely disintegrated. In roots grown at 30° C. the endodermis may persist longer than in roots grown at lower temperatures; this point will be discussed more at length in a later paragraph.

Metaxylem develops centripetally while the root is still very young, so that while the primary structures exist as such the center of the stele becomes solid xylem. The number of protoxylem groups varies somewhat with the variety of tobacco. Five and seven are common numbers in Maryland Broadleaf; four are fairly typical in both Burley types. The number, as stated previously, is often smaller in the minute branch-roots of all varieties. In Xanthia, for instance, two, three, four and five protoxylem groups have been observed.

Fasciation occurs occasionally in all varieties, and is common in Little Dutch. This condition may be manifested in all degrees from a slight widening of the stele in one plane to a form in which two separate steles

exist, connected only by parenchymatous tissue. In Little Dutch an intermediate type with fourteen protoxylem groups has been observed (Pl. LIX, fig. 24).

Shortly after the primary xylem has become complete, the fascicular cambium, between the protoxylem and protophloem elements, becomes active. Its growth pushes the protophloem groups into prominence, so that the stele takes on a scalloped periphery. Secondary wood is laid down simultaneously, next to the metaxylem. Soon the interfascicular cambium develops through division of pericyclic cells, and if these keep pace with the fascicular cambium, the stele again assumes a nearly circular cross section. Failure of the portions of the pericycle adjacent to the protoxylem groups to divide as rapidly as the fascicular cambium intensifies and prolongs the "scalloped" periphery of the stele. The formation of secondary wood often makes it very difficult to determine the original number of protoxylem groups because of a crowding of the tissues at the center of the root.

The time of initiation of pericyclic division varies with the variety of tobacco and with the temperature. At 20° C. the very susceptible Maryland Broadleaf and Susceptible Burley delay this action for a considerable length of time after the cambium has begun to divide. Thus a root of Maryland Broadleaf 1070 microns in diameter, with a stele 570 microns in diameter, showed large phloem bundles, a nearly complete cambium, and many large vessels in the xylem before the pericyclic cells had started to divide (Pl. LIX, fig. 25). In resistant varieties, such as Little Dutch, pericyclic activity is normally initiated at 20° C. almost as soon as the cambium begins to divide (fig. 24). When the pericycle of Susceptible Burley finally becomes the periphery of the root, however, a thicker layer of cork may be found (Pl. LIX, fig. 19), especially at 25° C., than in roots of Resistant Burley of the same age (fig. 18). The extremely resistant Xanthia exhibits a thick and perfect cork layer at 20° C. in roots of medium and large size.

After the primary cortex has disintegrated following pericyclic activity, the layer of cork, or phellem, one to several cell layers thick, lies at the root periphery. Just below this is the active phellogen. Next comes a rather thick layer of large-celled phelloderm, the three tissues just mentioned often being termed collectively the periderm. The boundaries between periderm and secondary phloem are indicated in a general way by the crushed remains of the primary phloem elements, recognizable as deep-staining, irregular lines between the phelloderm and phloem cells; this boundary is indicated also by the ends of the vascular rays in the phloem (figs. 18, 19).

Lenticels are not prominent in the cork layer in any variety of tobacco studied.

In the upper portion of the crown of the plant, where the internal

structure appears to be that of stem rather than of root, pericyclic activity may be delayed considerably. In fact, a periderm is developed normally only when the cortex is broken down at the periphery. In such cases a phellogen may develop locally just below the epidermis, or in the pericycle, or even deeper in the case of a wound. In extremely resistant Xanthia, however, both a subepidermal periderm and a periderm of pericyclic origin may exist in the crown at the same time, both exhibiting perfect cork layers several cells in thickness, even at 20° C.

Branch roots arise in the primary structure by active local division of the pericyclic cells just external to the protoxylem groups. The developing root tip ruptures or dissolves its way through the cortex. The outer layer of root-cap cells normally becomes suberized just before emergence. The position of branch roots in the stele is marked by broadly tapering vascular rays and by centripetal dips in the cambium, accompanied by a small amount of secondary xylem at these points (fig. 18).

In the susceptible varieties of tobacco, especially at 20° C., pericyclic activity in the branch roots is delayed relatively as long as it is in the main roots. This leaves a portion of ruptured cortex for a considerable time in the angle formed by the two roots. Even in Resistant Burley (figs. 22, 33) this is often true at 20° C., and in Susceptible Burley and very susceptible Maryland Broadleaf frequently at 25° C. In the very resistant Little Dutch and Havana No. 142, however, at 20° C. the cork layers of both main and branch roots develop almost simultaneously, resulting in a continuous peripheral cork sheath (Pl. LIX, figs. 20, 23). This manifestation of phellogen activity is especially conspicuous in Xanthia at 25° C. (Pl. LXI, fig. 34), but it occurs definitely at 30° C. even in the susceptible varieties of tobacco (Pl. LIX, fig. 21). A discussion of the exact nature of the stimulus or stimuli initiating phellogen formation is beyond the scope of this treatment. Priestley and Woffenden (1922) suggest sap pressure as an important factor.

Branch roots from a secondary structure and from a crown which has secondary vascular tissues, and from portions of the stem which may have been buried in the soil in transplanting, arise in the cambium-phloem region. Such branch roots have no connection with the primary xylem.

There is no true tap-root in the tobacco plant. The main root divides once or several times just below the crown, and the branches are at first about coequal. Later, however, one branch may outdistance the others and thus become an apparent tap-root.

Microchemical tests [4] show lignified xylem with pectic middle lamella; epidermal cells and cork cells both suberized and lignified but with little or no pectin or cellulose; cellulose in the cambium, young xylem, young phloem, phelloderm and uninjured cortical cells; pectin in old or broken-down phloem, suberized and lignified tissues.

[4] Microchemical tests used are those described by Chamberlain (1924).

In general, the root systems of all the varieties of tobacco studied are composed of fewer and larger roots at 20° C. than at higher temperatures. At 30° C. a large number of fine branch roots are formed, and all of these tend to remain small for long periods. Histological examination shows that this reduction in the size of the roots at 30° C. is due to relatively small production of phloem and periderm (Pl. LIX, fig. 21). Secondary xylem, while not so great in amount as in roots of the same age at lower temperatures, is not conspicuously lacking. Pericyclic division is apparently slow unless stimulated by outside agencies. The pericyclic regions in main and branch roots, however, develop almost simultaneously even in susceptible varieties, so that ruptures at branch root bases do not remain long unhealed. The cork layer is limited often to a single layer of cells, but this is conspicuously and almost perfectly circular in cross-sectional outline. A close examination of such cross sections reveals the fact that the endodermis, while almost completely collapsed, still exists as a perfect sheath about the outer layer of pericyclic cells. That the flattened endodermis is under considerable tension is demonstrated by spiral curling of the loose ends where it has ruptured in places, and by the fact that the underlying tissues bulge out slightly through these ruptures. Remaining cortical cells, endodermis, and the outer layer of pericyclic cells give evidence of being more heavily suberized than similar cells in low-temperature roots. Whether or not the continued presence of the collapsed endodermis exerts any considerable inhibition upon the growth of the stele because of its tension could not be determined definitely. In an infected root of Xanthia, however, an abnormal development of pericyclic cells beneath a heavily suberized endodermis was observed to crush the underlying phloem cells instead of rupturing the endodermis (Pl. LXI, figs. 37, 38). It seems probable, therefore, that the endodermis at 30° C., thus heavily suberized and under tension due to stelar growth, may both hinder further growth of the tissues within and act as a protective layer of considerable importance.

Pathological Histology

Penetration of suberized cell walls or cork cells in tobacco roots by a single, unaided hypha of *Thielavia basicola* has not been observed, although hyphae are often seen in close contact with the outer cork or suberized epidermal layers. Apparently penetration of such cell walls occurs only as the result of a sort of "mass action," where a weft of hyphae comes to lie in contact with the walls. In such cases the suberized and lignified walls slowly change to pectin-like substances, swelling markedly at the same time, probably due to the activity of accumulated enzymes secreted by the mass of mycelium which is aggregated in the immediate region. A number of hyphae then appear to "surge" through the weakened walls (figs. 4, 30), and the process may be repeated with the suberized walls in the next deeper cell layer.

The developing root tip and the adjacent region of elongation appear to be peculiarly resistant to attack by Thielavia. At least no instance of a successful attack on these tissues has been observed in root tips after their emergence from the cortex of the main root. Whether this resistance is due to the chemical content of the growing cells, to a resistant and perfect epidermis, or to an outstripping of the fungus through rapid growth, as suggested by Appel (1915), has not been determined. It is certain, however, that the epidermis is much more resistant to fungal invasion than are the cortical cells just below. Many instances have been observed where the cortex has suffered complete destruction by the fungus while the adjacent epidermal cells remain intact (figs. 4, 9, 13, 29, 30). The resistance of the rapidly growing regions may well be due to a combination of resistant epidermis and a growing away from the fungus. Elongating and embryonic root tissues, epidermal cells, and cork layers are no doubt subject to frequent accidental injury in the soil, due to root growth and other factors, and if the fungus happens to be in close proximity to the wounds, infection may result. This conclusion checks up well with the observation of Johnson and Hartman (1919) that infection is proportional to the amount of infestation in the soil, other factors being equal.

A critical stage in the life of the root with regard to infection appears to be that in which the epidermal layer has been ruptured and a cork layer has not yet been fully developed below by pericyclic activity. This stage occurs usually just as pericyclic activity is being initiated. It is accentuated in those varieties of tobacco in which pericyclic activity lags far behind cambial development. Here the epidermal layer may not be able to accommodate itself to stelar enlargement, and rupturing occurs without the protection of a cork layer beneath. At temperatures of the magnitude of 30° C., however, the epidermal layer appears to retain its resistance to fungal attack for relatively long periods, probably due to the slow stelar growth and to rapid suberization of deeper cortical layers as the outer layer ruptures and disintegrates (Pl. LIX, fig. 21). As mentioned also in an earlier paragraph, the heavily suberized endodermis, although entirely collapsed, may be a protective layer of some importance. The cortical cells intervening between the epidermal layer and the endodermis become entirely suberized only when exposed to soil constituents (Appel, 1906), but such a condition occurs only as the result of breaks in the cellular structure which may also allow the fungus to become strongly established between the cells, where it then dissolves its way further in spite of heavy suberization.

From direct observation, however, it appears that the most common point of fungal entrance is the region in which a branch root has ruptured its way through the primary cortex or through the secondary phelloderm. More than 50 percent of the lesions studied microscopically centered around the bases of branch roots, and many showed direct evidence that the fungus

had made its entrance through the ruptured tissues (figs. 2, 5, 7, 15, 16). Observations on infected root systems as a whole bear out the truth of this statement. Where lesions were young enough to be still definitely delimited, more than 50 percent of those examined were found to surround branch-root bases. This fact appears to be general with all the varieties of tobacco studied, and at all temperatures at which lesions caused definitely by Thielavia have been observed.

While fungal entrance seems to be effected in much the same ways in all varieties of tobacco, *host invasions* differ markedly. At 20° C. the very susceptible Maryland Broadleaf offers no apparent resistance to the progress of the fungal hyphae once they have entered the root (fig. 26). This applies to all tissues in the oldest and youngest roots. The normally occurring suberized and lignified tissues appear to slow down the progress of invasion when encountered, but only temporarily. Thus the fungus is often observed even in the xylem vessels of Susceptible Burley and very susceptible Maryland Broadleaf in old lesions at 20° C. Young branch roots in the infected cortex are readily parasitized, either by Thielavia or by a secondary invader (Pl. LVII, fig. 1).

Entirely different are the phenomena following fungus entrance into the roots of the extremely resistant Xanthia at 20° C. and above. Here fungal invasion of the tissues is followed consistently by the development of a phellogen which proliferates cork tissue at some distance in advance of the encroaching fungus. Even the primary cortex, commonly perfectly susceptible to fungal progress in most varieties, in Xanthia has been observed to develop a phellogen which definitely delimits the lesion with a cork layer (figs. 9, 27). This observation is interesting in the light of the investigations of Massart (1898, p. 50) and of Priestley and Woffenden (1922), who considered phellogen formation in the primary cortex as rare. In addition to this action on the part of the cortex, the pericycle, even though it may still be dormant beneath uninfected portions of the cortex, begins dividing actively under the lesion, laying down cork cells toward the fungus. The fact that the dormant pericycle is uniseriate makes it easy to determine exactly where division has occurred. This activity results in the production of a cork layer which is very successful in excluding the fungus from the vascular elements of the stele. In cases where the fungus spreads through the cortex so as to girdle the root completely, the entire pericycle becomes active in proliferating cork cells toward the invading mycelium. Only one case has been observed in which the fungus actually entered the stele of the extremely resistant Xanthia. Here the infection apparently took place through a branch root (identified by the presence of a broad vascular ray) when the main root was very young, since only primary tissues were infected (Pl. LXII, fig. 43). Serial transverse sections through the lesion show the development of a meristematic layer in the xylem parenchyma, the activity of which finally pushed the primary xylem *out of the root*, at

the same time walling out the fungus with cork tissue (figs. 8, 44). At one end of the lesion sections of primary xylem cells can be observed clinging to the periphery of the root which appears perfectly healthy above and below the lesion, although somewhat misshapen by abnormal cambial activity. This may explain why the crown and older roots of Xanthia were never found to show lesions, no actual infection having been observed in roots in which normal peripheral cork layers were present. Often, however, this layer showed the staining reaction with safranin characteristic of lesions, although no fungal hyphae could be demonstrated. The fungus may, of course, have been present in close proximity to the root while in the soil, but may have been washed off in the processes of fixing and imbedding. In such cases a slight centripetal dip of the periderm is the only indication of host reaction. All these reactions of Xanthia become most conspicuous at 25° C., although they are easily observable at 20° C. At 30° C. (figs. 10, 35) similar host reactions occurred occasionally, but since no fungal hyphae could be demonstrated in the host tissue, the cause of the reactions could not be determined definitely. This is keeping with the fact that no spores of Thielavia were found in apparent lesions at this temperature during gross observations of the root systems.

Those varieties of tobacco exhibiting resistance intermediate between that of Maryland Broadleaf and that of Xanthia show active reactions against Thielavia comparable with their demonstrated resistance at 20° C. Thus Susceptible Burley, only slightly more resistant than Maryland Broadleaf, shows but a low proportion of weak reactions, the majority of these being at the crown, which appears to be generally more resistant than roots, probably because of its greater age and development (see table 2 and figs. 15 and 39). Resistant Burley, apparently not very resistant at 20° C., shows moderate reactions with occasional strong reactions especially at the crown (figs. 5, 31). The very resistant Little Dutch and Havana No. 142 show strong reactions uniformly at 20° C. (figs. 3, 12).

At 25° C. all varieties of tobacco studied show either the inception of cork formation or an increased phellogen activity beneath lesions. Even the very susceptible Maryland Broadleaf shows a number of strong reactions at this temperature (Pl. LVII, fig. 2). Every lesion observed in Resistant Burley was accompanied by strong cork formation at 25° C. (figs. 6, 28, 32), and Susceptible Burley exhibited at least some phellogen activity in every instance (Pl. LVIII, fig. 16). In the very resistant Little Dutch and Havana No. 142 (figs. 4, 13, 29, 30, 40, 42) the only lesions showing no cork reactions at this temperature were in very young roots which were so badly disorganized as to lead one to suspect that they were not normal when attacked. The extremely resistant Xanthia, as described previously, shows few lesions and those occurring on small roots alone, with consistently strong, successful reactions at 25° C. (figs. 9, 27, 37, 38).

Only the varieties employed in Series II were successfully grown at

30° C. In these four cases the plants grown in infested soil made practically as good growth as those in sterilized soil (table 1). The slight differences observed might well be attributed to differences in soil composition and physical structure. Xanthia, as stated before, showed no lesions definitely caused by Thielavia at 30° C., although indications of fungal stimulation were observed (figs. 10, 35).

As shown in table 2, over 50 percent of the lesions sectioned were found

TABLE 2. *Summarized Results Showing the Number of Lesions in which Seven Varieties of Tobacco Grown at Different Temperatures Reacted to the Fungus Invasion by Formation of Cork*

Host	Temperature ° C.	Cork Formation in Lesions			Lesions at Branches
		Strong	Weak	None	
Maryland Broadleaf	20	0	0	14	8
	25	4	5	3	9
Susceptible Burley	20	0	5	8	8
	25	2	12	0	7
	30	5	1	0	2
Connecticut No. 38	30	5	0	0	?
Resistant Burley	20	3	10	2	11
	25	16	0	0	9
	30	5	0	0	2
Little Dutch	20	13	2	1	8
	25	10	0	1	10
Havana No. 142	20	8	1	1	4
	25	7	1	1	5
	30	7	0	0	4
Xanthia	20	10	0	0	3
	25	10	0	0	?
	30	3?	0	0	?

to be in connection with the bases of branch roots. Probably a much higher percentage of lesions actually occurred in such regions, but only the number of lesions cited showed this condition under microscopical examination of the prepared slides. It was commonly observed in the case of young branch roots that the fungus enters to a considerable depth through the ruptured cortical tissues. It then "backs up" and enters the unprotected stele of the branch root. Next it travels down the branch stele into the main root, disorganizing the tissues as it goes.

General Remarks

In general, *Thielavia basicola* enters the tobacco root and crown through natural or accidental wounds or by a "mass action" of aggregated hyphae. Probably only roots which have ceased to elongate are ever successfully attacked when in a normal living condition. Cell walls of cellulose or pectic material are penetrated readily by a single hypha (Pl. LXI, fig. 36), with little or no host reaction in early stages of penetration. Even normal nuclei have been observed in such cells in close proximity to the fungal

hypha. Some time after penetration the adjacent walls become pectic in nature, but it seems evident that actual penetration is largely, if not entirely, mechanical. In fact, it appears that neither host nor fungus exhibits the definite production of substances highly toxic to the opposite organism. Apparently enzymes from the fungus break down suberized and lignified tissues into pectin-like substances only when an aggregation of hyphae occurs in a localized region. The fungus appears to grow and sporulate as freely in a resistant host as in a susceptible one except for the checks interposed in the form of cork layers.

When hyphae encounter suberized or lignified walls, they are stopped temporarily. Continued growth of the hyphae soon fills the broken tissue, intercellular spaces, or pockets, with mycelium, probably identical with the "sclerotial masses" mentioned by Miss McCormick (1925). Following the massed condition of the fungus, the suberized and lignified walls change to pectin-like materials which appear to swell or "gelatinize." Cell walls thus affected give a characteristic deep-staining reaction with safranin. (This swelling of the cell walls should not be confused with host reaction against the progress of the fungus. The cells are already dead before being encountered. Careful observation will show that the walls of normal cork cells are not noticeably thicker than the cellulose walls of ordinary cells. The chief difference is in chemical composition.) Finally, the fungus breaks through the weakened walls (figs. 4, 30). In tissues of susceptible varieties, the fungus then continues to advance until the whole root is invaded and killed, followed by a rot associated with secondary invaders, generally soil bacteria.

In strongly resistant strains of tobacco, or in strains made resistant by exposure to high temperatures, the host tissue several layers of cells deeper than those actually invaded is stimulated to active cell division, forming a phellogen which further limits the progress of the fungus by proliferating cork cells toward the point of invasion. In the case of an extremely resistant primary root structure in such a variety as Xanthia, this reaction may take place even in the primary cortex. Usually, however, the reaction occurs first only in that part of the pericycle which lies directly under the lesion. In roots where little secondary thickening has taken place, it is impossible to distinguish pericycle from cambium as the cork-forming region, especially under deep lesions. (As a matter of fact, those portions of the cambium adjacent to the protoxylem groups, known as interfascicular cambia, are really pericyclic tissue.) The endodermis may become suberized under the lesion also, but shows no visible activity. If the fungus has girdled the whole root, the entire pericycle is stimulated to division (figs. 2, 32). In the semi-resistant varieties, this reaction fails to keep the fungus out of the stele in all cases, especially where the attack is made by a large weft of hyphae. In extremely resistant Xanthia, however, this "corking-out" has not been observed to fail in excluding the fungus from the stele.

In resistant roots showing secondary thickening, with little or no primary cortex remaining, an invasion through the cork layer is followed by the development of a new phellogen at a greater or less depth in the phelloderm (Pl. LXII, fig. 40). Tissues deeper in the root may then be involved gradually, until the phloem cells become active as the phellogen (fig. 41). Finally the cambium itself may become the phellogen (fig. 42). Where the fungus has entered the secondary xylem no further host reaction at that point has been observed.

Discussion

Few investigations of resistance in roots or subterranean plant parts to fungal invasion have been reported. The potato tuber is the classic example, and has been worked on by Appel (1906, 1915), Straňák (1918), Lutman (1919) and others. All these investigations show that normal cork and "wound-cork" are associated with resistance to fungal penetration and invasion. Weimer and Harter (1921) find that in sweet potato roots "wound-cork" is formed most rapidly at 33° C. and in a 95–100 percent relative humidity. They consider the "wound-cork" as a fairly efficient barrier to microörganisms.

Hawkins and Harvey (1919) suggest a rather different condition in potato tubers with regard to invasion. They find that internal tissues most resistant to mechanical puncture by a minute glass rod are found in varieties which are most resistant to attack by *Pythium debaryanum*. They believe that the cell walls alone are concerned in this mechanical type of resistance.

Tisdale (1917) reports a case of resistant action in roots which most nearly parallels the present investigation. He finds that roots of resistant flax infected with the flax-wilt fungus, *Fusarium lini*, produce cork layers between the infected outer portions and the vascular tissues. The flax-wilt fungus is a typical vascular parasite, while *Thielavia basicola* is chiefly a cortical invader. The disturbance of vascular functions is, however, of fundamental importance in both cases, whether as the result of plugging of vessels or as the result of root-rot, so a certain parallelism exists. Tisdale finds, moreover, that the fungus has an easy access to outer portions of the roots of resistant flax plants, and even of cabbage plants, but that it is unable, apparently, to invade vascular tissues of these plants. He suggests that substances may be present in resistant roots which may retard or weaken the fungus, thus adding to the effectiveness of the cork layer in excluding the fungus from the vascular system.

The study of root invasion by soil-inhabiting parasites is complicated by several factors. First, it is practically impossible to use soil infested with only the organism in question, especially in experiments conducted over a long period of time. In fact, the careful exclusion of all of the normal soil flora is likely to result in abnormal conditions in the development of the host as well as of the parasite. Second, root lesions cannot be

secured and fixed properly without some disturbance of the surface of the lesions. Third, the inception and progress of infection cannot be determined easily from time to time by direct observation of normally growing roots, as may be done with stem and leaf lesions. These factors make the study of fungus entrance into older root tissues very difficult. Furthermore, in the case of a root completely overcome, it is impossible to determine whether or not the fungus was the first or sole cause of the damage. Conversely, when no lesions occur, it is usually impossible to determine whether this is the result of host activity or of the absence of the fungus from close proximity to the root. In such cases cumulative data must have an important bearing in deciding the question.

Since epidermal cell walls and the walls of cork cells give identical microchemical reactions, it seems safe to conclude that penetration phenomena in connection with internal cork layers are identical with those in the external layers of the same nature. Thus the failure of individual hyphae of Thielavia to penetrate the internal cork layers unaided would indicate the same impotency of the fungus in the case of the epidermal cells and peripheral cork.

Further, the observations that some lesions show cork formation, while others in the same plant do not, need not be considered contradictory. Lesions unaccompanied by cork formation may have occurred in roots weakened by other factors prior to infection. Such roots may even have been dead or non-functional before the fungus entered them. In such cases actually observed, however, the unexpected lack of host reaction occurred only in very young roots. It may well be that a root needs to reach a certain stage of differentiation before cork formation can be initiated prematurely by special stimulation. Or, plants which appear to be semi-resistant may have root tissues in which the "corking-out" reaction to fungal stimulus is delicately balanced, and thus easily influenced one way or the other by local changes of environment. So, in a root system in which most of the lesions show cork formation, the presence of a few which do not may well be considered an abnormal condition. On the other hand, in a root system in which lesions are numerous and cork formation is absent in all of them, that condition must be considered the usual one in that particular plant.

The evidence presented in table 2 appears to leave no doubt as to the correct interpretation of the observed phenomena.

Observation shows that root tips and young elongating roots are not greatly concerned in tobacco root-rot because of their high resistance. Small absorbing roots are so numerous, and their functional life so short normally, that their destruction is not of great importance unless wholesale, which was obviously not the case in any of the present experiments. It is the preservation of large, conducting roots, then, that is of highest importance to the growth and normal functioning of the plant as a whole.

The rotting away of one large conducting root may render functionless a large proportion of the total number of absorbing roots, even though they themselves may be uninjured directly. It is of greatest importance to the plant, therefore, to have a mechanism for preventing or delaying the destruction of conducting roots, especially the larger ones. This requirement reaches its maximum at the crown of the plant where the roots join the stem. Moreover, since new roots commonly arise in large numbers at the crown to replace those which have been killed off below the crown, it is of the greatest importance that the crown be kept free from invasion.

It is certain that the very susceptible Maryland Broadleaf was unable to fulfill these requirements at 20° C. No visible reaction against the invading hyphae of Thielavia could be observed, and the plant made practically no growth over the entire period of the experiment. Lesions were numerous, especially at the crown, and much of the root system, even new roots just emerging from the crown, was invaded and killed.

On the other hand, the extremely resistant Xanthia showed a strong formation of cork beneath all lesions observed at 20° C., and all these were successful in excluding the fungus from the xylem elements. This phenomenon was accompanied by practically normal growth of the aërial portions. No lesions were found at the crown in any case, and but few on any of the roots growing in heavily infested soil.

Susceptible Burley, Resistant Burley, Little Dutch and Havana No. 142 show a gradient of host reactions, as expressed by cork formation, agreeing closely with the general vigor and empirical resistance of the respective plants.

In general, the results indicate that crowns and roots of large diameter, with their protective layers of peripheral cork, are very resistant to fungal attack. The points at which branch roots occur, however, often mark regions unprotected by cork layers, especially in susceptible varieties of tobacco in which pericyclic division is relatively late. This condition is intensified by low temperatures. The failure of the phellogens of main and branch roots to form rapidly a continuous sheath of peripheral cork commonly leads to the establishment of the fungus in the host tissues. When such infection occurs in resistant varieties a new phellogen may arise beneath the lesion, preventing further advance of the fungus by proliferating new cork cells as the old ones collapse. The entrance at the bases of branch roots is, however, much more frequent in susceptible varieties in which no new phellogen develops, and the fungus is then able to do much damage, even to the destruction of the entire root. The time of initiation of normal pericyclic activity, then, is an important factor in the resistance of tobacco to Thielavia, especially since this seems to be correlated with the ability of other tissues to develop phellogens.

The lack of numerous lesions at 30° C., accompanied by the formation of many branch roots and the slow development of pericyclic cork layers,

has not been satisfactorily explained. The most promising suggestion is that suberization of the surface layers is so rapid and complete that the fungus is unable to force an entrance into the tissues except in special, perhaps accidental, cases. It is also suggested that the endodermis, persisting as it does as a thin but strong sheath of collapsed cells about the pericycle, may play some part in this resistance to fungal penetration at 30° C. or thereabouts.

The enlargement of the root at the point of infection, mentioned casually by Gilbert (1909), may be explained here in the light of the present experiments. Without doubt that enlargement, with its scurfy surface, was a mass of proliferated cork filled with fungus mycelium. Since normal soil temperatures during the growing season often average about the degree necessary for the inception of premature cork formation even in the most susceptible varieties of tobacco, such enlargements of the root as points of infection might well have been found in any field on any variety studied. As a matter of fact, such enlargements have been observed on the infected older roots of Susceptible Burley at 25° C. in the present experiments.

It seems safe to conclude, therefore, that resistance in tobacco to the root-rot caused by *Thielavia basicola* is definitely correlated with the ability of the host to develop a cork layer beneath the point of infection. It seems true, also, that this reaction on the part of the host is accelerated by raising the temperature of the soil in which the plant is rooted, until at 26°–30° C., and above, all varieties of *Nicotiana tabacum* become resistant to attack by Thielavia. Therefore, it may be said that the lower the temperature at which a tobacco root may initiate normal, premature, or supplementary cork formation, the greater is the resistance of that root to *Thielavia basicola*.

This conclusion does not exclude the possibility, as suggested by Tisdale (1917) in the case of flax-wilt, that resistant tobacco roots may contain a chemical substance or character which so slows up initial fungal invasion as to permit time for the cork barrier to be laid down. It seems clear, however, that such an entity, if it exists, is not capable of excluding the fungus if unaided by cork formation.

Summary

1. Plants of seven varieties of tobacco showing a gradient from high susceptibility to high resistance have been grown in sterilized soil and in soil infested with *Thielavia basicola* at 20° C., 25° C., and 30° C. continuously for six weeks.

2. Microscopic examination of healthy roots taken from these plants show that susceptible varieties exhibit a lag in the initiation of pericyclic division behind cambial development, resulting in an unprotected primary cortex, and especially in an unprotected gap at the point where branch roots have ruptured through the cortex.

3. Cork formation is stimulated in susceptible varieties by relatively high temperatures, until, at 28° C. and above, the initiation of pericyclic activity occurs almost as early as in very resistant varieties at temperatures about 20° C. or below.

4. In Xanthia, the most resistant variety of tobacco studied, the pericycles of both main and branch roots show active division from the beginning of cambial growth through to maturity, and at all temperatures employed in the experiments.

5. Sections through lesions from plants grown in infested soil show a distinct correlation between resistance and cork formation in the tissues underlying the lesions, very susceptible varieties such as Maryland Broadleaf showing no cork formation at 20° C., but strong cork formation at 30° C.; while Xanthia, extremely resistant, shows strong cork formation at 20° C. (even xylem parenchyma becoming meristematic in deep lesions), with no lesions on mature roots and crowns, and no lesions on any portion of the plant at 30° C.

6. More than 50 percent of all lesions examined both macroscopically and histologically were found to occur at the bases of branch roots.

7. Epidermal layers and cork layers were found to be very resistant to mechanical penetration by the fungus, no case of direct penetration having been observed.

8. Root tips and adjacent elongating regions appear to be immune to attack by Thielavia, due perhaps to the resistance of their suberized epidermal cells, or to an "outstripping" of the fungus by growth, or to a combination of these factors.

9. The primary cortex of all varieties except Xanthia allows easy fungal invasion after penetration of the epidermal layer, no phellogen being developed by the cortical tissue in most cases.

10. Cork cell walls and other suberized and lignified walls are slowly changed to pectin-like substances in the presence of masses of fungus mycelium, probably due to the accumulation of enzymes in localized regions, the walls thus acted upon swelling to an abnormal thickness and becoming so weakened that they allow the penetration of deeper tissues by hyphae from the mycelial mass lying adjacent.

11. Fungal entrance stimulates premature local pericyclic division in young roots of resistant plants in advance of invasion.

12. All secondary tissues of resistant roots except dead xylem elements are able under fungal stimulation to produce cork cells by developing a phellogen in advance of invasion.

13. Neither host nor parasite appears to produce substances particularly toxic to the other.

14. The crowns of tobacco plants and portions of stems buried in transplanting exhibit the "corking-out" reaction even more markedly than do the roots.

15. The rapidity with which a tobacco plant can initiate and continue cork formation beneath a lesion is an accurate criterion of its resistance to Thielavia.

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LITERATURE CITED

Appel, O. 1906. Zur Kenntnis des Wunderverschlusses bei den Kartoffeln. Ber. Deutsch. Bot. Ges. **24**: 118–122.

——. 1915. Disease resistance in plants. Science, n. ser. **41**: 773–782.

Chamberlain, C. J. 1924. Methods in plant histology.

Gilbert, W. W. 1909. The root-rot of tobacco caused by *Thielavia basicola*. U. S. Dept. Agr. Bur. Plant Ind. Bull. 158.

Hawkins, L. A., and Harvey, R. B. 1919. Physiological study of the parasitism of *Pythium debaryanum* Hesse on the potato tuber. Jour. Agr. Res. **18**: 275–297.

Johnson, J. 1916*a*. Host plants of *Thielavia basicola*. Jour. Agr. Res. **7**: 289–300.

——. 1916*b*. Resistance in tobacco to the root-rot disease. Phytopathology **6**: 167–181.

——., **and Hartman, R. E.** 1919. Influence of soil environment on the root-rot of tobacco. Jour. Agr. Res. **27**: 41–86.

——., **and Milton, R. H.** 1919. Strains of White Burley tobacco resistant to root-rot. U. S. Dept. Agr. Bull. 765.

Jones, L. R. 1917. Soil temperatures as a factor in phytopathology. Plant World **20**: 229–237.

Lutman, B. F. 1919. Resistance of potato tubers to scab. Vt. Agr. Exp. Sta. Bull. 215.

McCormick, Florence A. 1925. Perithecia of *Thielavia basicola* Zopf in culture and the stimulation of their production by extracts from other fungi. Conn. Agr. Exp. Sta. Bull. **269**: 539–554.

Massart, J. 1898. La cicatrisation chez les vegetaux. Mém. Acad. Roy. Belgique **57**: 1–68.

Peglion, V. 1897. Marciume radicale delle piantine di tabacco causato dalla *Thielavia basicola* Zopf. Cent. Bakt. Abt. II, **3**: 580–584.

Priestley, J. H., and Woffenden, L. M. 1922. Physiological studies in plant anatomy. New Phytol. **21**: 252–268.

Rawlins, T. E., and Johnson, J. 1925. Cytological studies of the mosaic disease of tobacco. Amer. Jour. Bot. **12**: 19–32.

Straňák, F. 1918. Beiträge zur histologischen und physiologischen Erforschung der bakteriellen Krankheit der Gefässbündel der Kartoffelknollen. Cent. Bakt. Abt. II, **48**: 520–543.

Tisdale, W. H. 1917. Flaxwilt: a study of the nature and inheritance of wilt resistance. Jour. Agr. Res. **11**: 573–605.

Weimer, J. L., and Harter, L. L. 1921. Wound-cork formation in the sweet potato. Jour. Agr. Res. **21**: 637–647.

EXPLANATION OF PLATES

PLATES LVII AND LVIII

The figures in these plates are semidiagrammatic representations of sections of infected tobacco roots showing resistant reactions of various strains to *Thielavia basicola* at different temperatures. The outlines of the tissues are accurate since they were drawn with the aid of a camera lucida, under a magnification of 30 diameters. Stippled areas represent invaded regions; cross-ruled areas designate cork layers, the innermost boundaries of which represent phellogens, or, where, the line extends beyond cork tissues, pericycle; the innermost line locates the cambium in each case. Unshaded areas within the peripheral boundaries of the roots are normal tissues.

FIG. 1. Cross section of a young root of very susceptible Maryland Broadleaf showing only primary structures, grown in infested soil at 20° C. The fungus has entered all portions of the cortex, encountering no cork layer, penetrating the pericycle and even the cambium and xylem. A branch root has been parasitized before emergence from the cortex.

FIG. 2. Cross section of a moderately old root of very susceptible Maryland Broadleaf grown in infested soil at 25° C. Here the fungus has been "corked out" at the periphery by a broad cork layer except at the point where a branch root departed from the stele. The fungus has penetrated through the cambial region at this point. Contrast with figure 1.

FIG. 3. Cross section of a very young root of very resistant Little Dutch grown in infested soil at 20° C. The fungus has penetrated only a limited region, but the pericycle, still dormant in uninvaded portions of the root, has proliferated a layer of cork cells under the lesion, considerably in advance of fungal invasion. Contrast with figure 1.

FIG. 4. Longitudinal section of a young root of very resistant Little Dutch grown in infested soil at 25° C. The pericycle has become active in forming a cork sheath which effectually protects the stele. Note the portions of resistant epidermis bordering the infected tissue; also a point at the upper right where a mass of hyphae is breaking through the first line of cork cells.

FIG. 5. Portion of a cross section of an old root of Resistant White Burley, grown in infested soil at 20° C. The fungus has entered the host through ruptured tissues at a branch root base. Active cork formation in the main root has excluded the fungus from the stele, but lack of pericyclic division in the branch root has allowed the fungus to penetrate its xylem.

FIG. 6. Portion of a cross section of a crown of Resistant Burley grown in infested soil at 25° C. The fungus has penetrated deeply into the cortical tissue of the host, but the active pericycle has proliferated cork cells far in advance of invasion, protecting the stele.

FIG. 7. Cross section of a fairly young root of Resistant Burley grown in infested soil at 30° C. A layer of cork surrounds the entire root. Infection apparently took place through a branch root base, spreading gradually around the root. Cork formation has been initiated deeper and deeper in the periderm and even in the xylem parenchyma, effectually corking out the fungus from the stele.

FIG. 8. Cross section of a young root of extremely resistant Xanthia grown in infested soil at 20° C. Penetration of the stele took place at an early stage through the base of a branch root, as revealed by other sections in the same series. The primary xylem of the main root was actually invaded, and the adjacent secondary xylem parenchyma was stimulated to activity on the side opposite the branch root, resulting in the expulsion of the infected primary tissue (darkly stippled) from the root. A cork layer has developed beneath this entire region, protecting the root from further invasion, and the cambium has again formed a complete sheath about the remains of the secondary xylem. See also figures 43 and 44 for other sections from this same series.

FIG. 9. Cross section of a young root of extremely resistant Xanthia grown in infested soil at 25° C. The pericycle is proliferating cork cells beneath the lesion, and the primary cortex has formed two radiating phellogens far in advance of invasion. (In the radiating areas in the cortex, the irregular radiating lines represent the actual directions of the cross walls.) Note the uninvaded epidermis surrounding the greater part of the infected tissue.

FIG. 10. Cross section of a young root of extremely resistant Xanthia grown in infested soil at 30° C. Only primary structures appear. The fungus has entered only the broken-down peripheral cortical cells, but the living cortical cells just below have reacted by laying down cross walls, thus initiating a phellogen. (The cells of the cortex are accurately drawn with the aid of a camera lucida, the pericycle and endodermis being represented by a narrow ring just outside the cambium.)

FIG. 11. Cross section of a young root of susceptible Connecticut No. 38 grown in infested soil at 30° C. The pericycle shows normal cork formation throughout its circumference, but an abnormally thick layer of cork has developed under the invaded portion of the cortex.

FIG. 12. Portion of a cross section of a crown of very resistant Havana No. 142 grown in infested soil at 20° C. A heavy invasion of the cortex has resulted in the stimulation of the pericycle underlying the lesion to cork formation, effectually protecting the phloem and deeper tissues. Compare with figures 6 and 17.

FIG. 13. Cross section of a young root of very resistant Havana No. 142 grown in infested soil at 25° C. The fungus, apparently entering through a break in the epidermis, has spread rapidly through the primary cortex. The pericycle has been stimulated to cork formation far in advance of invasion. Note the uninvaded epidermis. Compare with figures 9 and 11.

FIG. 14. Cross section of a very young root of very resistant Havana No. 142 grown in infested soil at 30° C. Fungal penetration at a branch root base has stimulated the underlying pericycle to active division, resulting in a cork layer which protects the main stele.

FIG. 15. Portion of a cross section of an old root of Susceptible White Burley grown in infested soil at 20° C. A late infection by the fungus at the base of a branch root has resulted in the penetration of the main stele since the fungus encountered no new cork layer. Contrast with figure 5.

FIG. 16. Cross section of an old root of Susceptible Burley, including a branch root base, grown in infested soil at 25° C. Fungal entrance probably occurred at the branch root base. Lack of pericyclic activity in the branch root has allowed fungal penetration deep into the stele, and at one point the parasite has entered the stele of the main root. Contrast with figure 7.

FIG. 17. Portion of a cross section of a crown of Susceptible Burley grown in infested soil at 30° C. The cortical tissue has been invaded heavily, but a cork layer of pericyclic origin has effectually blocked further advance by the fungus. Compare with figures 6 and 12.

PLATE LIX

The figures in this plate are photomicrographs of sections of normal tobacco roots of several varieties grown at different temperatures. Magnifications are given with the figures.

FIG. 18. Cross section of an old root of Resistant Burley grown at 25° C. Note the thin peripheral cork layer; also the broad vascular ray in the upper portion of the stele. × 10.

FIG. 19. Cross section of an old root of Susceptible Burley grown at 25° C. Note the thickness of the cork sheath, and compare with figure 18. × 12.

FIG. 20. Cross section of an old root of very resistant Havana No. 142 grown at 20° C. This is a typical protected branch root base of a resistant strain of tobacco at a low temperature. Note the continuity of cork tissue across the angles at the branch root base. × 30.

FIG. 21. Cross section of a moderately old root of Susceptible Burley grown at 30° C. This is a typical protected branch root base of a susceptible strain of tobacco at a high temperature. Compare with figure 20. × 30.

FIG. 22. Cross section of a fairly old root of Resistant Burley (a semi-resistant strain) grown at 20° C. Note that pericyclic development in the main root has not resulted in the entire disappearance of the primary cortex; also that the cork layer of the main root ends abruptly at the branch root base in which no pericyclic division has taken place. This is a typical unprotected root base in a semi-resistant strain of tobacco at a low temperature. × 20.

FIG. 23. Cross section of a moderately old root of very resistant Little Dutch grown at 20° C. Note that even at this low temperature pericyclic activity has been initiated in the branch root, resulting in a protected root base. Contrast with figure 22. × 15.

FIG. 24. Cross section of a young root of very resistant Little Dutch grown at 20° C. Note the "fasciation." Pericyclic division has been initiated at an early stage, resulting in a cork layer almost encircling the stele, and especially conspicuous at the branch root base. × 25.

FIG. 25. Cross section of a young root of very susceptible Maryland Broadleaf grown at 20° C. Note that in this root, about the same age as that shown in figure 24, little or no division has occurred in the cells of the pericycle, although secondary growth due to cambial activity has proceeded to a considerable extent. Contrast with figure 24. × 30.

PLATE LX

The figures in this plate are photomicrographs of sections of infected tobacco roots of several varieties grown at different temperatures. The magnifications are given with the figures.

FIG. 26. Cross section of a young root of very susceptible Maryland Broadleaf grown in infested soil at 20° C. The fungus has invaded all portions of the cortex, and has even broken into the xylem in places. No cork formation has occurred. See also figure 1, and contrast with figure 29 which shows an infected resistant root of about the same age. × 40.

FIG. 27. Cross section of a young root of extremely resistant Xanthia grown in infested soil at 25° C. Note that pericyclic division has occurred actively under the lesion. Note also the phellogens in the primary cortex on either side of the lesion, radiating outward from the endodermis. See also figure 9. × 45.

FIG. 28. Portion of a cross section of an old root of Resistant Burley (semi-resistant) grown in infested soil at 25° C. A few invading fungal hyphae have been almost completely surrounded by a phellogen which has arisen in the phelloderm and phloem. Such "strands" are of common occurrence in resistant roots which have been invaded sparingly. × 40.

FIG. 29. Cross section of a young root of very resistant Little Dutch grown in infested soil at 25° C. Note that the pericycle has been stimulated to cork formation under the lesion, while it is still dormant in normal portions of the root. Note also the uninvaded epidermis surrounding the lesion. × 35.

FIG. 30. Longitudinal section of a portion of a young root of very resistant Little Dutch grown in infested soil at 25° C. Note the long line of uninvaded cork tissue in the upper portion of the section, and places where the fungus is breaking through the cork layer on the opposite side. This demonstrates the penetration of cork layers by aggregated masses of fungal hyphae in localized points. See also figure 4. × 35.

FIG. 31. Cross section of a young root of Resistant Burley (semi-resistant) grown in infested soil at 20° C. The invading fungus has stimulated pericyclic activity in two places under lesions. Phellogens are being initiated to some extent in the cortex. × 50.

FIG. 32. Cross section of a fairly young root of Resistant Burley grown in infested soil at 25° C. The fungus has almost girdled the root, but is being successfully resisted by a cork layer. The two small, dark regions lying in the cork tissue on opposite sides of the root are infected protoxylem groups which have been pushed outward by meristematic activity in the secondary xylem parenchyma. × 35.

PLATES LXI AND LXII

The figures in these plates, pen-and-ink drawings made with the aid of a microprojector, represent cross sections of normal and infested tissues illustrating the host reactions in several varieties of tobacco roots under different environmental conditions. Magnifications are given in the descriptions.

FIG. 33. Portion of a cross section of a normal, moderately old root of Resistant Burley (semi-resistant) grown at 20° C., illustrating a typical unprotected branch root base. The pericycle of the main root has not developed far; the primary cortex is still in place for the most part. Pericyclic division has not started in the branch root. The result of this lag in pericyclic activity is the prolonged presence of a mass of ruptured cortical tissue at the base of the branch root, suitable for easy fungal invasion and establishment. × 70.

FIG. 34. Portion of a cross section of a normal, moderately old root of extremely resistant Xanthia grown at 25° C. This section shows a peripheral cork layer continuous across the region at the base of a branch root. The primary cortex has practically disappeared, due largely to the pushing out of the cork layer resulting from early pericyclic division. In such a case the fungus encounters a resistant cork layer at all points in the periphery of the root. × 70.

FIG. 35. Cross section of a very young root of Xanthia (extremely resistant) grown in infested soil at 30° C., showing only primary structures. Note the three protoxylem groups, the undifferentiated phloem, the uniseriate pericycle (stippled), the endodermis with Casparian strips on the radial walls, and the regular cortex. Several layers of cells at the periphery of the cortex have disappeared, and a cortical phellogen is forming, probably due to fungal stimulation. See also figure 9. × 150.

FIG. 36. Portion of a cross section of the invaded cortex of Resistant Burley grown at 25° C. The out-curved condition of the cell wall which has been penetrated by a hypha of Thielavia seems to indicate that the puncture was mechanical, in part at least. × 150.

FIG. 37. Cross section of a very young root of extremely resistant Xanthia grown in infested soil at 25° C. Note that the pericycle, still undivided under normal portions of the cortex, has formed a cork layer several cells thick underlying the invaded cortical tissue. Note also that the primary cortex has formed phellogens radiating out from the endodermis, far in advance of fungal invasion. (The solid black cell inclusions represent portions of fungal hyphae.) See also figure 27. × 70.

FIG. 38. Portion of the cross section shown in figure 37, more highly magnified. The endodermis with its Casparian strips is conspicuous. Note that the cork layer is thickest in the region where the fungus has penetrated deepest; note also the crushed phloem under this cork layer, and lack of secondary growth due to cambial activity. × 150.

FIG. 39. Portion of a cross section of an infected old root of Susceptible Burley grown at 20° C. The fungus has penetrated the peripheral cork layer and has invaded the phelloderm and phloem freely, encountering no further resistance in the form of a cork layer. × 70.

FIG. 40. Portion of a cross section of an invaded old root of very resistant Little

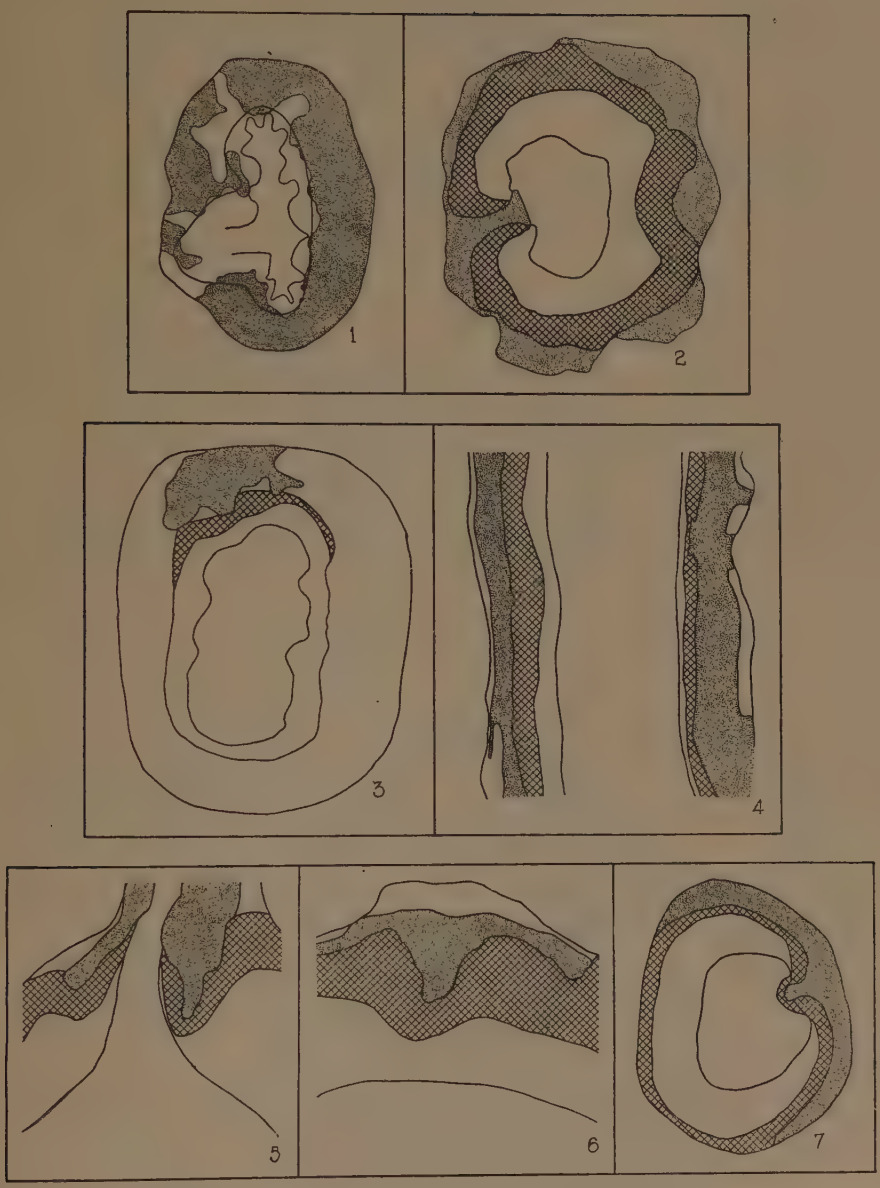
Dutch grown at 25° C. Here the phelloderm has developed a definite phellogen under the point where the fungus has broken through the phellem. Note that the new phellogen is formed far in advance of fungal invasion. × 70.

FIG. 41. Portion of a cross section of an invaded, fairly young root of Connecticut No. 38 (susceptible at low temperatures), grown at 30° C. Beneath a deep portion of the lesion the phloem cells have been stimulated into activity, causing them to become a part of the phellogen. × 150.

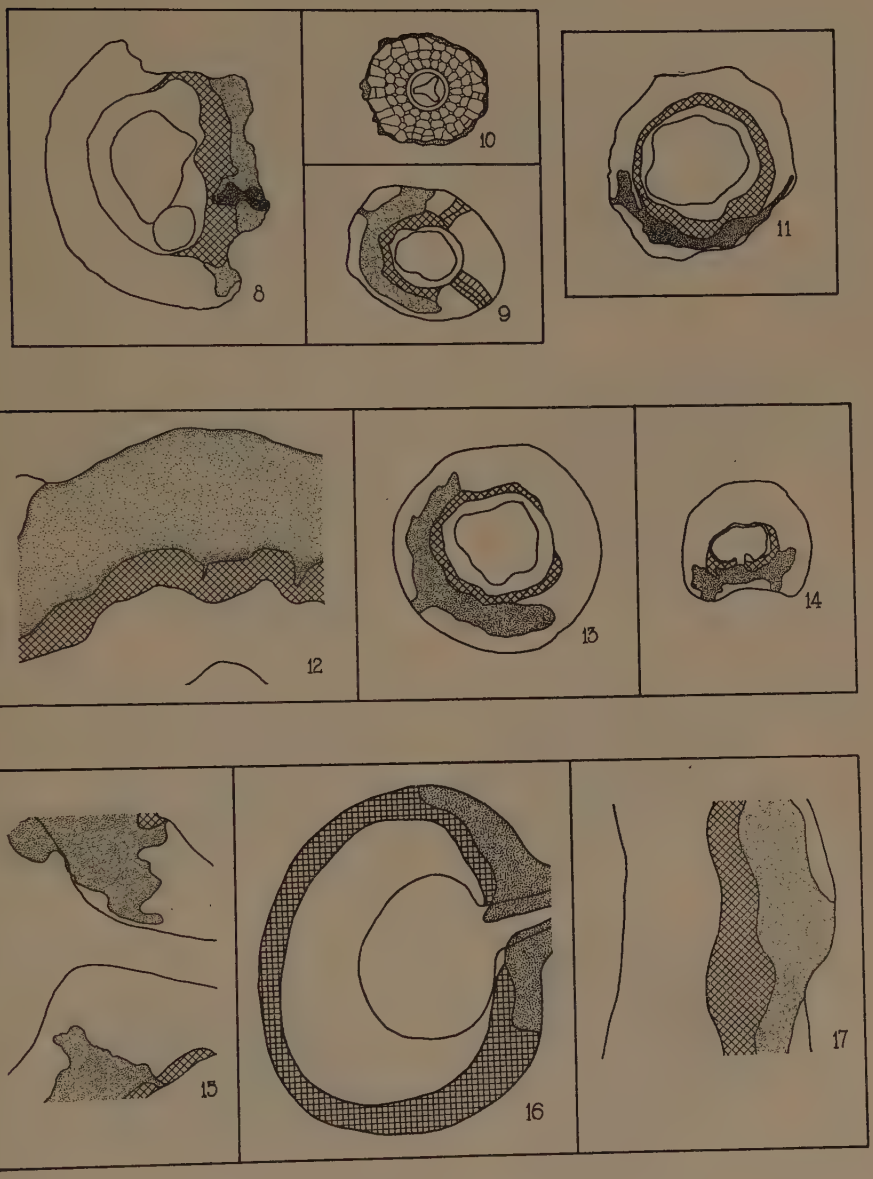
FIG. 42. Portion of a cross section of a fairly young root of very resistant Little Dutch grown at 25° C. Under a deep portion of the lesion the cambium has become the cork-forming meristem. In the upper left portion of the figure the pericyclic region is still the seat of cork formation. × 70.

FIG. 43. Portion of a cross section of a rather young root of extremely resistant Xanthia grown at 20° C. The primary xylem is invaded, and the xylem parenchyma surrounding it has become meristematic. In the upper portion are shown two elements of a protoxylem group which have been separated from the main mass of infected primary xylem. × 150.

FIG. 44. Portion of a cross section from another level of the lesion shown in section in figure 43. Here the greater part of the invaded primary xylem has been pushed toward the periphery of the root due to the active division of cells of the secondary xylem parenchyma. See also figure 8 for another section in the same series which shows the infected primary xylem almost removed from the root. × 150.



CONANT: HISTOLOGICAL STUDIES OF RESISTANCE



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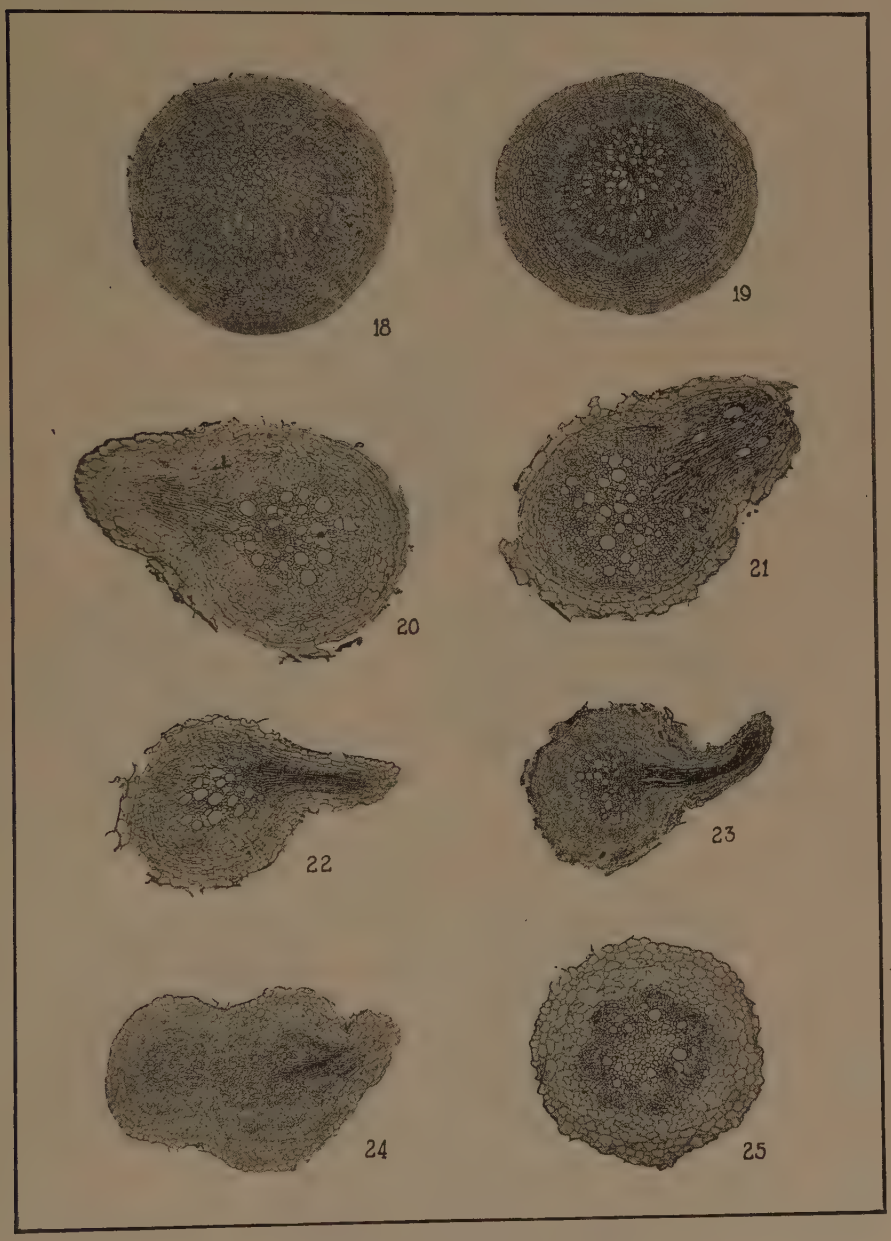

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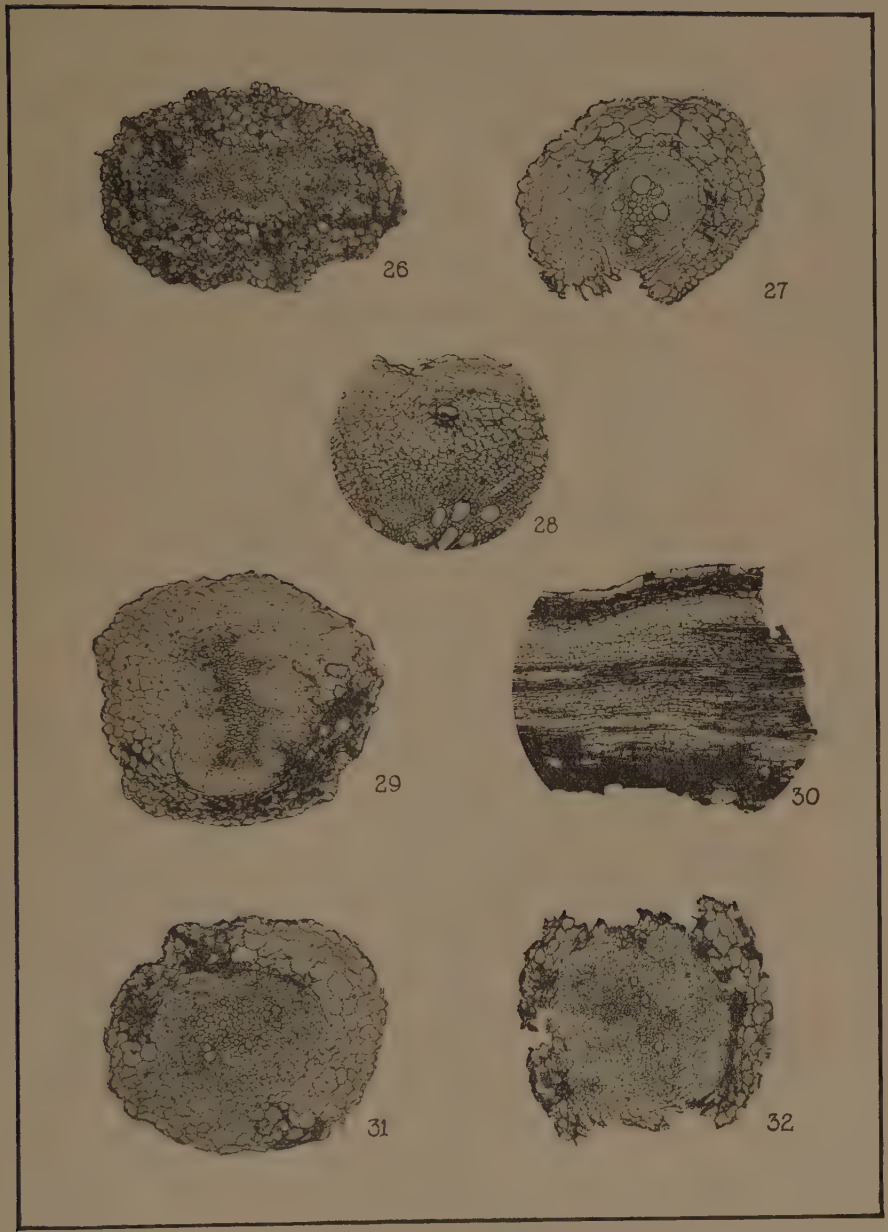

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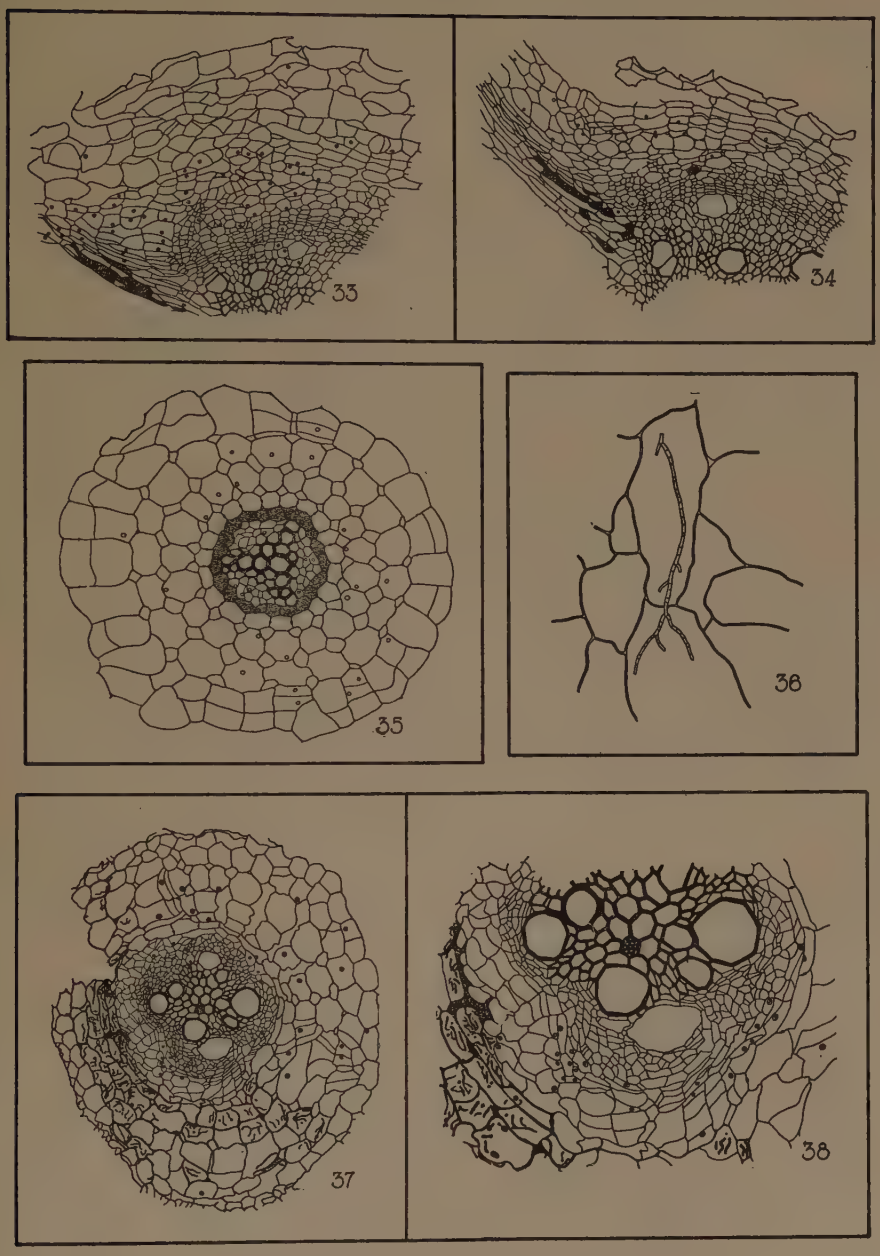

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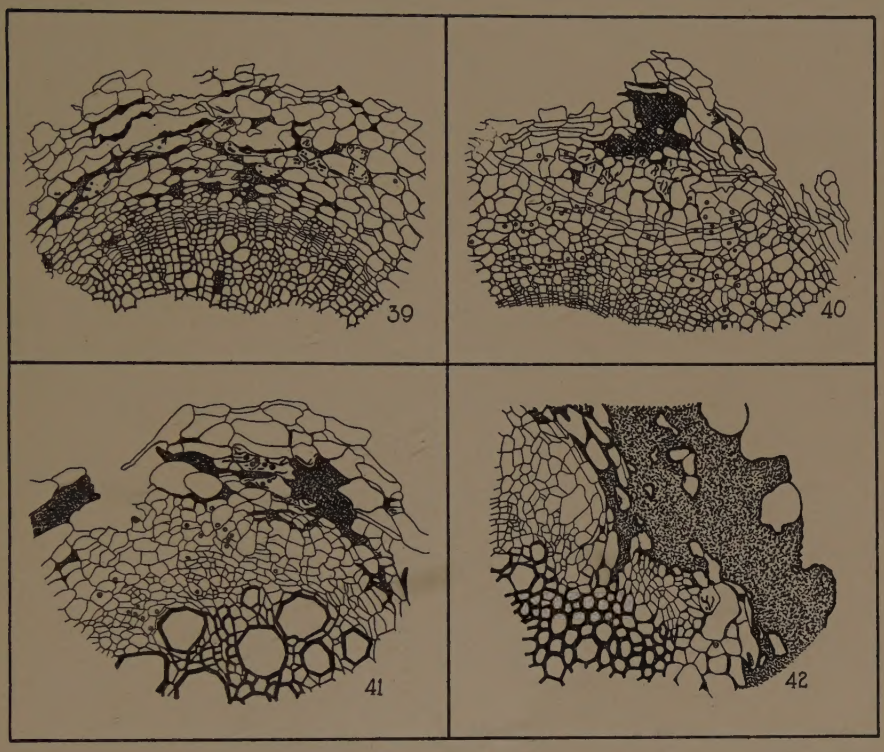

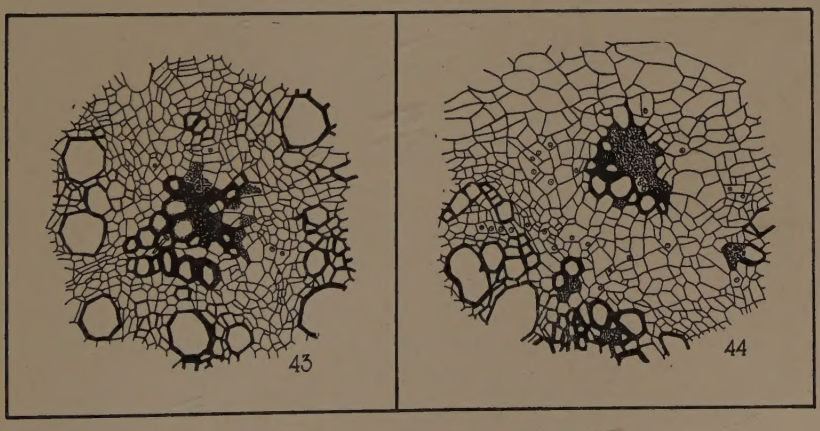

CONANT: HISTOLOGICAL STUDIES OF RESISTANCE